PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference ABL-008-PCT	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/BE 03/00190	International filing date *(day/month/year)* 07.11.2003	Priority date *(day/month/year)* 08.11.2002
International Patent Classification (IPC) or both national classification and IPC C07K16/42		
Applicant ABLYNX N.V.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 8 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 6 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☒ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
01.06.2004	22.02.2005
Name and mailing address of the international preliminary examining authority: European Patent Office - Gitschiner Str. 103 D-10958 Berlin Tel. +49 30 25901 - 0 Fax: +49 30 25901 - 840	Authorized Officer Alconada Rodríguez, Telephone No. +49 30 25901-326

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/BE 03/00190

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-103 as originally filed

Claims, Numbers

1-51 received on 07.12.2004 with letter of 01.12.2004

Drawings, Sheets

1-14 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

☐ the language of publication of the international application (under Rule 48.3(b)).

☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

☐ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☒ furnished subsequently to this Authority in written form.

☒ furnished subsequently to this Authority in computer readable form.

☒ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☒ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

☐ the description, pages:

☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application,

   ☒ claims Nos. 1-14, 46-51 (in part), 17-45 (complete) and 1-8, 15 and 16 (with respect to industrial applicability)

   because:

   ☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (specify):

   ☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☒ no international search report has been established for the said claims Nos. 1-14, 46-51 (in part), 17-45 (complete) and 1-8, 15 and 16 (with respect to industrial applicability)

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the Standard.

   ☐ the computer readable form has not been furnished or does not comply with the Standard.

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees, the applicant has:

   ☒ restricted the claims.

   ☐ paid additional fees.

   ☐ paid additional fees under protest.

   ☐ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

☐ complied with.

☐ not complied with for the following reasons:

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

☐ all parts.

☒ the parts relating to claims Nos. 1-14 and 46-51 (in part) and 15-16 (complete) .

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	-
	No:	Claims	1-16, 46-51
Inventive step (IS)	Yes:	Claims	-
	No:	Claims	1-16, 46-51
Industrial applicability (IA)	Yes:	Claims	9-14,46-51
	No:	Claims	-

2. Citations and explanations

see separate sheet

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Claims 1-8, 15 and 16 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(I) PCT).

Re Item IV

Lack of unity of invention

This authority agrees with the International Search Authority (ISA) in that the present application contains 11 inventions which are not so linked as to form a single general inventive concept, as required by Rule 13.1 PCT for the following reasons.

The application relates to different antibody sequences. The common concept underlying the plurality of antibody sequences is that they are all single domain antibodies. Single domain antibodies are known in the prior art. For instance, Muyldermans, S. (2001) Reviews in molecular biotechnology, 74:277-302 describes methods for the isolation of single domain antibodies from camelidae (see figure 5) and provides references to prior art documents which report the isolation of single domain antibodies against different antigens (see table 1). In light of this prior art the above mentioned common concept is not novel and the problem underlying the present application can be redefined as the provision of additional single domain antibodies. The sequences identified in inventions 1 to 11 are different solutions to this problem. Due to the fact that single domain antibodies are known in the prior art and due to the fact that no other technical feature can be distinguished which, in the light of the prior art, could be regarded as a special technical feature in the sense of Rule 13.2 PCT due to the essential differences in the primary structures and on the nature of the of antigens recognised by the claimed single domain antibodies, there is no single general inventive concept underlying the plurality of claimed inventions of the present application in the sense of Rule 13.1 PCT. Consequently, the application does not meet the requirements of unity of invention as defined in Rules 13.1 and 13.2 PCT.

The applicant has requested the preliminary examination to be carried out on invention 2 (anti-TNF-alpha camelidae VHH antibodies and uses thereof) corresponding to present claims 1-14, 46-51 (in part) and 15 and 16 (complete) (previous claims 11-24 and 58-63 (in part) and 25 and 26 (complete)).

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following document:

D1: WO 91/02078 A (PEPTIDE TECHNOLOGY LTD) 21 February 1991 (1991-02-21)

D2: MUYLDERMANS S: "SINGLE DOMAIN CAMEL ANTIBODIES: CURRENT STATUS" REVIEWS IN MOLECULAR BIOTECHNOLOGY, ELSEVIER, AMSTERDAM,, NL, vol. 74, no. 4, June 2001 (2001-06), pages 277-302, XP001057480 ISSN: 1389-0352

D1 discloses monoclonal antibodies against human TNF-alpha, including single domain antibodies (see page 4 lines 15-20) and the uses thereof for the treatment of diseases where it is desired to inhibit TNF-alpha activity (see page 4, lines 6-14). The single domain Ab to which D1 relates are those as defined by Ward et al. (Nature, 1989, 341:544-546) which relate to conventional ScFv consisting of the covalently-linked VH and VL regions of a monoclonal antibody, wherein the single domain antibodies of the present application relate to the variable region of the heavy chain of a camelidae antibody, which is naturally devoid of light chains. It appears that, even if the intention of the applicant was to obtain antibodies consisting of a single chain, the fact that the term "single domain antibodies" has been used in the prior art to relate to scFv antibodies results in a lack of novelty for the subject-matter of **claims 9-14 and 47-51**, which relate to the single domain antibodies as such, as well as for the subject-matter of **claims 1-8**, which relate to the uses of said antibodies.

In addition, the IPEA is of the opinion that the application lacks inventive step for the following reasons. D1, which can be considered as closest prior art, discloses different types of antibodies specific for TNF-alpha and their use as inhibitors of TNF-alpha activity.

The present application differs from the subject-matter of D1 in that the application uses anti-TNF-alpha camelidae VHH antibodies. Thus, the problem to be solved by the present application can be summarised as the provision of alternative monospecific anti-TNF-alpha antibodies. The problem is solved by the general VHH anti-TNF-alpha antibodies of claims 1-15 and by the specific VHH antibodies of SEQ ID NO:12-14 as defined in claim 16. The solution provided in the present application can not be considered as involving an inventive step since it was already known at the time of filing the application that camelidae VHH antibodies provide a convenient alternative to other types of monospecific antibodies (see D2, pages 280-290). These antibodies, due to their relative simple structure, show certain functional, technological and physico-chemical properties (see page 291, left-hand column, first paragraph) which would make then advantageous over other types of monospecific antibodies. Thus, the skilled person, when confronted with the above problem, would attempt to obtain camelidae anti-TNFalpha VHH antibodies as described in D2, thus arriving to the subject-matter of **claims 1-15.**

Moreover, the specific VHH molecules of SEQ ID NO:12-14 could only be considered to involve an inventive step if they show some unexpected or surprising properties. The applicants have provided evidence that the VHH antibody of SEQ ID NO:12 (TNF3E) shows an increased stability in the presence of pepsin (example 5), that the TNF3E antibody can be orally administered (example 6) and that the bivalent construct of SEQ ID NO:14 (consisting of TNF3E covalently linked to TNF3F) can be used for the treatment of chronic colitis (example 7). However, none of these properties provided in the examples can be considered as surprising with respect to what could be expected from the known properties of camelidae VHH antibodies described in the prior art (see list on page 291, left-hand column in D2). Thus, the skilled person, when obtaining camelidae anti-TNFalpha VHH according to the combined teaching of D1 and D2, would inevitably arrive to VHH molecules, which, if not identical to those of TNF3E and TNF3F, would show exactly the same properties. Therefore, no inventive step can be acknowledged for the subject-matter of claim 16, as far as it relates to the monomeric camelidae VHH of SEQ ID NO:12 and 13. Likewise, the use of two different VHH molecules to prepare bivalent mono- or bispecific VHH is also known from D2 (see figure 6) and thus, it would also be obvious for the skilled person to combine the non inventive monovalent anti-TNFalpha VHHs to obtain bivalent constructs as that of SEQ ID NO:14. Thus, **claim 16**, as far as it relates to the camelidae VHH of SEQ ID NO:14 is also devoid of an inventive step.

Claims 1-8 relates to different methods for the therapeutic administration of the anti-TNFalpha VHH to a subject. All the different methods relate to nothing else than a shopping list of all possible administration ways that are known from common pharmacology handbooks and for which no inventive step can be acknowledged if they do not involve the administration of a new and inventive compound. Likewise, **claims 9-14** relate to medical uses of the non-inventive polypeptides, where the compound is further described by its ability to pass through different biological barriers without being inactivated. These claims are identical in scope to claims 1-8 and are therefore also considered as to lack an inventive step.

CLAIMS (retyped)

1. A method for delivering an anti-target compound to a subject for the treatment of a disorder without being inactivated by administering thereto a polypeptide construct comprising one or more single domain antibodies directed against said target.

2. A method according to claim 1 wherein said target is located in the gut system, and said a polypeptide construct is delivered orally.

3. A method according to claim 1 wherein said target is located in vaginal and/or rectal tract, and said a polypeptide construct is delivered to the vaginal and/or rectal tract.

4. A method according to claim 1 wherein said target is located in nose, upper respiratory tract and/or lung, and said a polypeptide construct is delivered to nose, upper respiratory tract and/or lung.

5. A method according to claim 1 wherein said target is located in intestinal mucosa, and said a polypeptide construct is delivered orally.

6. A method according to claim 1 wherein said target is located in the tissues beneath the tongue, and said a polypeptide construct is delivered to the tissues beneath the tongue.

7. A method according to claim 1 wherein said target is located in the skin, and said a polypeptide construct is delivered topically.

8. A method according to claim 1 wherein said target is in, or accessible via the blood, and said a polypeptide construct is delivered orally, is delivered to the vaginal and/or rectal tract, is delivered nasally by inhalation though the mouth or nose, to the tissues beneath the tongue, or topically.

9. A polypeptide construct comprising at least one single domain antibody directed against a target, for use in treating, preventing and/or alleviating the symptoms of disorders which are susceptible to modulation by an anti-target therapeutic compound that is able to pass through the gastric environment without being inactivated.

10. A polypeptide construct comprising at least one single domain antibody directed against a target for use in treating, preventing and/or alleviating the symptoms of disorders which are susceptible to modulation by an anti-target therapeutic compound that is able to pass through the wall of the intestinal mucosa without being inactivated

11. A polypeptide construct comprising at least one single domain antibody directed against a target for use in treating, preventing and/or alleviating the symptoms of disorders which are susceptible to modulation by an anti-target therapeutic compound that is able to pass through the wall of the nose, upper respiratory tract and/or lung without being inactivated

12. A polypeptide construct comprising at least one single domain antibody directed against a target for use in treating, preventing and/or alleviating the symptoms of disorders which are susceptible to modulation by an anti-target therapeutic compound that is able to pass through the wall of virginal and/or rectal tract without being inactivated

13. A polypeptide construct comprising at least one single domain antibody directed against a target for use in treating, preventing and/or alleviating the symptoms of disorders which are susceptible to modulation by a therapeutic compound that is able to pass through the tissues beneath the tongue without being inactivated

14. A polypeptide construct comprising at least one single domain antibody directed against a target for use in treating, preventing and/or alleviating the symptoms of disorders which are susceptible to modulation by a therapeutic compound that is able to pass through the skin without being inactivated

15. A method according to any of claims 1 to 8 or polypeptide construct according to any of claim 9 to 14, wherein said target is TNF-alpha and the disorder is inflammation.

16. A method or polypeptide according to claim 15, wherein a single domain antibody corresponds to a sequence represented by any of SEQ ID NOs: 12 to 14.

17. A method according to any of claims 1 to 8 or polypeptide construct according to any of claim 9 to 14, wherein said target is CEA and the disorder colon cancer.

18. A method according to any of claims 1 to 8 or polypeptide construct according to any of claim 9 to 14, wherein said target is EGFR and the disorder is any of head, neck, lung and colon cancer.

19. A method or polypeptide construct according to claim 18, wherein a single domain antibody corresponds to a sequence represented by any of SEQ ID NOs: 23 to 44

20. A method according to any of claims 1 to 8 or polypeptide construct according to any of claim 9 to 14, wherein said target is an antigen of *Helicobacter pylori* and the disorder is any of indigestion, gastritis.

21. A method according to any of claims 1 to 8 or polypeptide construct according to any of claim 9 to 14, wherein said target is an antigen of *Mycobacterium tuberculosis* and the disorder is tuberculosis.

22. A method according to any of claims 1 to 8 or polypeptide construct according to any of claim 9 to 14, wherein said target is an antigen of *influenza* virus and the disorder is flu.

23. A method according to any of claims 1 to 8 or polypeptide construct according to any of claim 9 to 14, wherein said target is an antigen of MMP and the disorder is cancer.

24. A method or polypeptide construct according to claim 23, wherein a single domain antibody corresponds to a sequence represented by any of SEQ ID NOs: 15 to 22

25. A method according to any of claims 1 to 8 or polypeptide construct according to any of claim 9 to 14, wherein said target is antigen of IFN-gamma and the disorder is any of cancer, transplant rejection, auto immune disorder.

26. A method or polypeptide construct according to claim 25, wherein a single domain antibody corresponds to a sequence represented by any of SEQ ID NOs: 45 to 70

27. A method according to any of claims 1 to 8 or polypeptide construct according to any of claim 9 to 14 wherein said target is any of antigen of *Helicobacter pylori*, antigen of *Mycobacterium tuberculosis*, antigen of *influenza* virus.

28. A polypeptide construct comprising at least one single domain antibody directed against an internalising cellular receptor, and at least one single domain antibody directed against a therapeutic target.

29. A polypeptide construct comprising at least one single domain antibody directed against an internalising cellular receptor, and at least one therapeutic polypeptide or agent.

30. A polypeptide construct according to claims 28 or 29 wherein said internalising cellular receptor is Epidermal Growth Factor receptor.

31. A polypeptide construct according to claim 30 wherein a single domain antibody directed against an internalising cellular receptor corresponds to a sequence represented by SEQ ID NO: 23 to 44.

32. A polypeptide construct according to claims 28 or 29 wherein said internalising cellular receptor is any of LDL receptor, FGF2r, ErbB2r, transferring receptor, PDGr, VEGr, or PsmAr.

33. A polypeptide construct according to any of claims 28 to 32 wherein a single domain antibody directed against a therapeutic target, is directed against PDK1.

34. A polypeptide construct according to claim 33 for use in treating cancer

35. A polypeptide construct according to any of claims 28 to 32 wherein a single domain antibody directed against a therapeutic target is directed against any of GSK1, Bad, caspase and Forkhead.

36. A polypeptide construct according to claim 35 for use in treating cancer.

37. Method for delivering an anti-target therapeutic compound to the interior of a cell comprising administering to a subject a polypeptide construct according to any of claims 28 to 36.

38. Method for delivering an anti-target therapeutic compound to the interior of a cell without being inactivated comprising administering to a subject a polypeptide construct according to any of claims 28 to 37.

39. A method according to claim 38 wherein said cell is located in the gut system, and said polypeptide construct is delivered orally.

40. A method according to claim 38 wherein said cell is located in vaginal and/or rectal tract, and said polypeptide construct is delivered to the vaginal and/or rectal tract.

41. A method according to claim 38 wherein said cell is located in nose, upper respiratory tract and/or lung, and said polypeptide construct is delivered to nose, upper respiratory tract and/or lung.

42. A method according to claim 38 wherein said cell is located in intestinal mucosa, and said polypeptide construct is delivered orally.

43. A method according to claim 38 wherein said cell is located in the tissues beneath the tongue, and said polypeptide construct is delivered to the tissues beneath the tongue.

44. A method according to claim 38 wherein said cell is located in the skin, and said polypeptide construct is delivered topically.

45. A method according to claim 38 wherein said cell is in, or accessible via the blood, and said polypeptide construct is delivered orally, to the vaginal and/or rectal tract, nasally, by inhalation though the mouth or nose, to the tissues beneath the tongue, or topically.

46. A polypeptide construct according to any of claims 9 to 14, 28 to 36, or a method according to any of claims 1 to 8, 15 to 27, 37 to 45, wherein the single domain antibodies are humanized *Camelidae* VHHs.

47. A polypeptide construct according to any of claims 9 to 14, 28 to 36, 46, or a method according to any of claims 1 to 8, 15 to 27, 37 to 46, wherein said single domain antibody is an homologous sequence, a functional portion, or a functional portion of an homologous sequence of the full length single domain antibody.

48. A polypeptide construct according any of claims 9 to 14, 28 to 36 , 46 and 47 or a method according to any of claims 1 to 8, 15 to 27, 37 to 47, wherein the polypeptide

construct is an homologous sequence, a functional portion, or a functional portion of an homologous sequence of the full length polypeptide construct.

49. A polypeptide construct according to any of claims 9 to 14, 28 to 36, 46 to 48 or a method according to any of claims 1 to 8, 15 to 27, 37 to 48 wherein said single domain antibodies are *Camelidae* VHHs.

50. A nucleic acid encoding a polypeptide construct according to any of claims 9 to 14, 28 to 36, 46 to 49.

51. A composition comprising a polypeptide construct as defined in any of the preceding claims, together with a pharmaceutical carrier.